4772

Acid Enhancement of *Clostridium botulinum* Inhibition in Ham and Bacon Prepared with Potassium Sorbate and Sorbic Acid

C. N. HUHTANEN*, J. I. FEINBERG, H. TRENCHARD and J. G. PHILLIPS[1]

Eastern Regional Research Center[2], Philadelphia, Pennsylvania 19118

(Received for publication March 14, 1983)

ABSTRACT

The effectiveness of combinations of sorbic acid and other acids (hydrochloric, phosphoric, acetic, citric, lactic and succinic) on *Clostridium botulinum* inhibition in comminuted ham and nitrite-free bacon was studied. These acids, when added to ham to give similar pH's, did not significantly inhibit the organism, but when acetic or citric acid was added with sorbic acid, inhibition was greater than with sorbic acid alone. The acids were less effective in inhibiting *C. botulinum* when added to sorbic acid-containing bacon. A study of the effectiveness of three levels of potassium sorbate (0.10, 0.26 or 0.52%) or sorbic acid (0.08, 0.20 or 0.40%) and two levels of phosphoric acid (0.04 or 0.08%) in comminuted ham showed that the highest levels of sorbate or sorbic acid were sufficient to inhibit toxin production when incubated at 30°C for 180 d. The same degree of *C. botulinum* inhibition was afforded by 0.26% sorbate with 0.08% H_3PO_4 or by 0.20% sorbic acid with 0.04% H_3PO_4. These differences were probably due to the higher pH obtained with sorbate.

Sorbic acid and potassium sorbate have been shown to be effective inhibitors of *Clostridium botulinum* in various products including meat. Tompkin et al. (*12*) showed that 0.1% potassium sorbate increased the time for uncured sausage to become toxic after inoculation with *C. botulinum* spores. Ivey et al. (*7*) and Sofos et al. (*11*) reported a delay in toxicogenesis from *C. botulinum* in potassium sorbate-treated bacon, and Ivey and Robach (*6*) found it to be effective in canned comminuted pork. Sofos et al. (*10*) demonstrated the effectiveness of a combination of sorbic acid and nitrite in preventing toxin production by *C. botulinum* in chicken frankfurter emulsions. Huhtanen and Feinberg (*4*) also showed sorbic acid to be effective in inhibiting *C. botulinum* in chicken and turkey frankfurters and emulsions, especially after acidification with phosphoric acid. Huhtanen et al. (*5*) found that the antibotulinal efficacy of sorbic acid in bacon increased on acidification.

Because of the greater efficacy of the undissociated acid, it would be desirable to decrease the pH of products where sorbic acid or its potassium salt might be used. The experiments we report here were designed to determine the effectiveness of various acids as acidifying agents for inhibiting *C. botulinum* toxin production in comminuted ham and bacon. The effect of several levels of phosphoric acid and potassium sorbate in ham on *C. botulinum* inhibition was also studied.

MATERIALS AND METHODS

Comminuted meat

Fresh ham shoulders were obtained from a local meat wholesaler and were ground three times through a 0.25-in. plate. In order to simulate a shelf-stable, nitrite-free product, 3.8% NaCl and 2% sucrose were added. The added salt caused an increase in brine concentration to 5.9%, based on the analyzed water content of 61.05%. Although the added salt was slightly higher than normally used [most luncheon meats have between 3.0 and 3.4% added salt (*3*)], the brine concentration was insufficient to prevent outgrowth of *C. botulinum* spores in a nitrite-free, normal pH system (*9*).

Nitrite-free bacon was prepared by a local processor. The bacon was frozen, ground, mixed and packaged in 2-kg amounts in heat-sealable plastic bags. These were frozen after sealing under vacuum, then, when needed, were thawed quickly under running tap water. The ingredients were blended thoroughly by hand mixing and packed into 208 × 107 aluminum tab cans (75 g per can, five cans per treatment). The cans were sealed under 18 to 20 in. of vacuum in a Rooney canner (Rooney Machine Co., Bellingham, WA), heated for 30 min to 68°C center temperature in a water bath and, after cooling rapidly in tap water, were temperature-abused at 30°C for periods up to 180 d or until swelling occurred.

Acidification

Preliminary titrations were made of 100-g portions of comminuted ham or bacon in 200 ml of H_2O. Acids were slowly added with stirring until the desired target pH of 5.7 was reached. These figures were used in calculating the necessary amounts of acids to be added to the meat. The actual acid additions, in meq, for 1170-g portions of the comminuted ham were: 7.85 HCl; 16.34 H_3PO_4; 8.77 acetic acid; 13.67 citric acid; 8.38 lactic acid; or 11.22 succinic acid. Sorbic acid was added to a final concentration of 0.10%. The acid additions, in meq, to 1150-g portions of comminuted bacon were: 30.12 HCl; 52.25 H_3PO_4; 29.27 acetic acid;

---

[1]*Consulting Statistician, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, PA 19118.*
[2]*Agricultural Research Service, U.S. Department of Agriculture.*

37.11 citric acid; 31.04 lactic acid; or 34.07 succinic acid. Eight ml of a sorbate slurry (15 g potassium sorbate, 56 ml 1.05 N HCl, and 54 ml H_2O) at pH 5.7 was added where specified. This gave a final concentration (expressed as sorbic acid) of 0.06%, a level chosen from previous work (*5*) as being insufficient by itself to appreciably inhibit *C. botulinum*. Water was added where needed to maintain equality of fluid additions.

Spores

A mixture of 12 type A and 8 type B spores were prepared by the method of Huhtanen and Feinberg (*4*). The same strains were used with the exception of 1 type B strain (FSQS #5) which sporulated poorly. The spore mixture contained equal numbers of spores of all strains; the final concentration was 400 per g of comminuted meat.

pH measurements

One volume of distilled water was added to 10 g of meat in a small beaker; the mixture was triturated with a glass stirring rod and after standing 1 h the pH was determined with an Orion model 601 pH meter.

Toxin tests

Selected swollen or non-swollen cans were assayed for toxin by adding 2 volumes of gelatin phosphate buffer (pH 6.2) to the contents of the can in a Whirl-Pak bag. This was mixed thoroughly by hand kneading and was centrifuged at 10,000 × *g* after standing overnight in a refrigerator. Two mice were injected with 0.5 ml each of the extract and were examined for 3 d. The extracts were considered to be toxin positive when both mice died with typical symptoms. If one mouse died, the test was repeated; this was rarely the case, however.

RESULTS AND DISCUSSION

The effect of the acids in ham, when added at levels giving similar pH values, is shown in Table 1. Although the target pH was 5.70, after processing the actual pH values were closer to 5.80, due perhaps to release of intracellular components on heating. The acids alone had no effect on numbers of swollen cans during the first week [for statistical purposes, using five cans per treatment ($P<0.05$), a difference of four cans would be needed to show a significant difference (*1*)]. At 1 wk there were no swollen or toxic cans with sorbic acid alone or with the acids; at 2 wk three of the sorbic acid cans were toxic. At 4 wk only the sorbic acid-acetic acid treatment (no toxic cans) was significantly different from the sorbic acid treatment alone (four toxic cans).

An experiment with nitrite-free bacon (Table 2), showed that although the acids prevented can swelling, all samples were toxic. Potassium sorbate added to bacon did not inhibit *C. botulinum,* as all cans were swollen and toxic in 10 d. When citric acid was added with sorbic acid, an apparently significantly degree of antibotulinal activity appeared during the first week; however, on subsequent incubation there was no difference in numbers of toxic cans when potassium sorbate was added by itself or with citric acid.

The effect of two levels of phosphoric acid (0.04 and 0.08% wt/wt) and three levels of potassium sorbate (0.10, 0.26 and 0.52%) or three levels of sorbic acid (0.08, 0.20 and 0.40%) was determined in comminuted ham (Table 3). Potassium sorbate alone at 0.52% inhibited *C. botulinum* for the duration of the 180-d abuse period, i.e., there were no swelled or toxic cans. This is in contrast with our previous results (*4*) with poultry frankfurters where only slight antibotulinal activity was produced by this level of sorbate. At 0.26%, although the mean swell time (MST) was delayed to 72 d (the controls had a MST of 15 d), all cans were toxic. This was also true when 0.20% sorbic acid was used. When equivalent quantities of sorbic acid were used

TABLE 1. *Effect of acids on sorbate inhibition of* C. botulinum *in ham*[a].

		Incubation time (wk)[b]											
		1				2				4			
		Swollen cans				Swollen cans				Swollen cans			
Addition[c]	pH	Mean	Range	No./5	Cans toxic/5	Mean	Range	No./5	Cans toxic/5	Mean	Range	No./5	Cans toxic/5
None	6.07	7	7	1	5	10	9-12	5	5	ND[d]	ND		
HCl	5.92	-	-	0	5	12	10-14	4	5	ND	ND		
H_3PO_4	5.87	-	-	0	3	-	-	0	4	ND	ND		
Acetic acid	5.92	-	-	0	2	12	10-13	3	4	ND	ND		
Citric acid	5.88	-	-	0	3	14	13-14	2	5	ND	ND		
Lactic acid	5.94	-	-	0	4	11	10-12	2	5	ND	ND		
Succinic acid	5.92	-	-	0	3	13	13-14	3	5	ND	ND		
Sorbic acid (0.10%)	5.93	-	-	0	0	-	-	0	3	29	29	1	4
$NaNO_2$ (120 μg/g)	5.89	-	-	0	0	-	-	0	0	24	24	1	0
Sorbic + HCl	5.81	-	-	0	0	-	-	0	0	-	-	0	2
Sorbic + H_3PO_4	5.80	-	-	0	0	-	-	0	0	-	-	0	2
Sorbic + acetic	5.80	-	-	0	0	-	-	0	0	33	33	1	0
Sorbic + citric	5.78	-	-	0	0	-	-	0	1	20	16-24	2	3
Sorbic + lactic	5.76	-	-	0	0	-	-	0	2	24	19-28	2	4
Sorbic + succinic	5.75	-	-	0	0	13	13	1	2	-	-	0	3

[a]Comminuted fresh pork shoulders, 3.4% NaCl and 2% sucrose added.
[b]Incubated at 30°C.
[c]See text.
[d]ND, not done.

TABLE 2. *Effect of acids on sorbate inhibition of* C. botulinum *in bacon*[a].

		Incubation time (d)[b]											
		10				17				31			
		Swollen cans				Swollen cans				Swollen cans			
Addition[c]	pH	Mean	Range	No.	Cans toxic	Mean	Range	No./5	Cans toxic/5	Mean	Range	No./5	Cans toxic/5
None	6.40	10	10	10/10	10/10								
HCl	5.91	10	10	1/5	5/5	12	11-12	4	5	ND[d]	ND		
H_3PO_4	5.73	-	-	0/5	5/5	12	11-13	5	5	ND	ND		
Acetic acid	5.85	-	-	0/5	5/5	13	12-13	5	5	ND	ND		
Citric acid	5.82	-	-	0/5	5/5	14	13-17	5	5	ND	ND		
Lactic acid	5.75	-	-	0/5	5/5	14	13-17	5	5	ND	ND		
Succinic acid	5.70	10	10	1/5	5/5	12	11-14	5	5	ND	ND		
Potassium sorbate	6.30	10	10	15/15	15/15								
$NaNO_2$ (120 µg/g)	6.35	-	-	0/5	0/5	-	-	0	0	-	-	0	0
Sorbate + HCl	5.75	-	-	0/5	2/5	-	-	0	5	20	17-21	5	5
Sorbate + H_3PO_4	5.83	-	-	0/5	4/5	17	17	1	5	20	17-21	4	5
Sorbate + acetic	5.85	-	-	0/5	1/5	17	17	1	5	25	24-26	5	5
Sorbate + citric	5.74	-	-	0/5	0/5	-	-	0	5	26	23-31	5	5
Sorbate + lactic	5.66	-	-	0/5	1/5	-	-	0	4	28	25-31	2	4
Sorbate + succinic	5.65	-	-	0/5	3/5	-	-	0	5	20	17-23	5	5

[a]Nitrite-free comminuted bacon, see text.
[b]Incubation at 30°C.
[c]See text.
[d]ND, not done.

TABLE 3. *Effect of phosphoric acid on sorbic acid or potassium sorbate inhibition of* C. botulinum *in comminuted ham.*

Additions				Swell time (d)			
Potassium sorbate (%)	Sorbic acid (%)	H_3PO_4 % (wt/wt)	pH	Mean	Range	No. cans swollen/ No. tested	No. can toxic/ No. tested
-	-	-	5.78	15	12-18	5/5	5/5
0.10	-	-	5.78	35	24-39	5/5	5/5
0.26	-	-	5.75	72	68-83	5/5	5/5
0.52	-	-	5.82	>180	-	0/5	0/5
-	0.08	-	5.73	42	33-55	5/5	5/5
-	0.20	-	5.61	99	88-114	3/5	5/5
-	0.40	-	5.70	>180	-	0/5	0/5
-	-	0.04	5.76	13	12-13	5/5	5/5
-	-	0.08	5.64	21	18-24	5/5	5/5
0.10	-	0.04	5.77	43	29-57	5/5	5/5
0.10	-	0.08	5.73	56	41-64	4/5	4/5
0.26	-	0.04	5.71	177	-	1/5	4/5
0.26	-	0.08	5.69	>180	-	0/5	2/5
0.52	-	0.04	5.73	>180	-	0/5	0/5
0.52	-	0.08	5.70	>180	-	0/5	0/5
-	0.08	0.04	5.59	70	67-77	4/5	5/5
-	0.08	0.08	5.35	66	60-76	4/5	4/4
-	0.20	0.04	5.46	>180	-	0/5	0/5
-	0.20	0.08	5.42	>180	-	0/5	0/5
-	0.40	0.04	5.45	>180	-	0/5	0/5
-	0.40	0.08	5.32	>180	-	0/5	0/5
Control with 156 µg/g $NaNO_2$				93	83-102	5/5	5/5

instead of the potassium salt, the pH values, as expected, were slightly reduced and there was a slight increase in the MST. Again, however, all cans were toxic except those with 0.40% sorbic acid. Phosphoric acid by itself, at a concentration of 0.08%, was only slightly effective in increasing the MST, even though the pH was lowered to 5.64. This was a lower pH than that obtained with 0.40% sorbic acid (5.70) which gave complete protection. When phosphoric acid was added to ham containing 0.10% potassium sorbate, there was an increase in MST greater than that exhibited by either ingredient alone. All cans tested, however, were toxic. Addition of 0.04% phosphoric acid to meat containing 0.26% potassium sorbate increased the MST to 177 d with only one can swelling. This can and three unswollen cans were toxic. When 0.08% phosphoric acid was used in conjunction with 0.26% potassium sorbate, no cans swelled but two were toxic at the end of the abuse period.

The lowest level of sorbic acid in the ham (0.08%) when acidified with phosphoric acid, showed considerably increased MSTs in the cans compared to those of the sorbic acid alone; however, all cans tested were toxic. With 0.20% sorbic acid together with 0.04 or 0.08% phosphoric acid, there were no swelled or toxic cans during the abuse period. By contrast, 156 μg/g sodium nitrite alone, although exhibiting a much longer MST than the control, showed toxin in all five cans.

Our results (Table 2) indicate that neither potassium sorbate alone or in combination with any of the acids tested was as effective as 120 μg/g $NaNO_2$ in inhibiting *C. botulinum* in comminuted bacon. This level of $NaNO_2$ added directly before subjecting to abuse temperature is higher than that normally expected after processing, since several days elapse before finished bacon is distributed and subjected to possible abuse conditions. At this time the actual nitrite content would be less than half the starting amount (*2*).

Toxin production in the absence of overt gas production is commonly found in experiments of this kind. Our results indicate that this phenomenon may be more prevalent when meat is acidified. Similar results in culture media (*8*) were recently reported. Our results indicate, however, that gas production is merely delayed by the acidification; after further incubation, other cans from the same treatments became swollen. An exception to this was with H_3PO_4 in comminuted ham (Table 1) where toxic cans were found throughout the incubation period in the absence of swelling.

Our data indicate that sorbic acid or potassium sorbate can be combined with phosphoric acid to provide effective antibotulinal protection in comminuted ham. This is similar to our results with chicken or turkey frankfurter emulsions (*4*). For protection for 180 d at 30°C, a combination of 0.52% potassium sorbate and 0.04% H_3PO_4 was required. The same degree of protection was afforded by 0.20% sorbic acid plus 0.04% H_3PO_4 or by 0.40% sorbic acid alone.

ACKNOWLEDGMENTS

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

REFERENCES

1. Agricultural Research Service. 1967. Table of exact probabilities for 2 × 2 contingency tables. ARS Publication No. 2015, U.S. Department of Agriculture, Washington, DC.
2. Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, T. V. Kueper, R. W. Johnston, D. A. Kautter, and O. J. Kolari. 1974. Effect of sodium nitrite on toxin production by *Clostridium botulinum* in bacon. Appl. Microbiol. 27:733-737.
3. Komarik, S. L., D. K. Tressler, and L. Long (eds.). 1974. Food products formulary, vol. 1. Meats, poultry, fish, shellfish. AVI Publishing Co., Westport, CT.
4. Huhtanen, C. N., and J. Feinberg. 1980. Sorbic acid inhibition of *Clostridium botulinum* in nitrite-free poultry frankfurters. J. Food Sci. 45:453-457.
5. Huhtanen, C. N., F. B. Talley, J. Feinberg, and J. G. Phillips. 1981. Flavor and antibotulinal evaluation of sorbic acid-containing bacon. J. Food Sci. 46:1796-1800.
6. Ivey, F. J., and M. C. Robach. 1978. Effect of sorbic acid and sodium nitrite on *Clostridium botulinum* outgrowth and toxin production in canned comminuted pork. J. Food Sci. 43:1782-1785.
7. Ivey, F. J., K. J. Shaver, L. N. Christiansen, and R. B. Tompkin. 1978. Effect of potassium sorbate on toxicogenesis of *Clostridium botulinum* in bacon. J. Food Prot. 41:621-625.
8. Montville, T. J. 1983. Dependence of *Clostridium botulinum* gas and protease production on cultural conditions. Appl. Environ. Microbiol. 45:571-575.
9. Roberts, T. A., and M. Ingram. 1973. Inhibition of *Clostridium botulinum* at different pH values by sodium chloride and sodium nitrite. J. Food Technol. 8:467-475.
10. Sofos, J. N., F. F. Busta, K. Bhothipaksa, and C. E. Allen. 1979. Sodium nitrite and sorbic acid effects on *Clostridium botulinum* toxin formation in chicken frankfurter-type emulsions. J. Food Sci. 44:668-675.
11. Sofos, J. N., F. F. Busta, K. Bhothipaksa, C. E. Allen, M. C. Robach, and M. W. Paquette. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on *Clostridium botulinum* toxin production in commercially prepared bacon. J. Food Sci. 45:1285-1292.
12. Tompkin, R. B., L. N. Christiansen, A. B. Shaparis, and H. Bolin. 1974. Effect of potassium sorbate on salmonellae, *Staphylococcus aureus, Clostridium perfringens* and *Clostridium botulinum* in cooked, uncured sausage. Appl. Microbiol. 28:262-264.